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Improved metabolic response after 16 weeks of calorie-restricted low-glycaemic index diet and metformin in impaired glucose tolerance subjects

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Abstract

Aim: **This study analyzed the metabolic effects of dietary advice to follow calorie-restricted low-glycaemic index diet with metformin in overweight / obese impaired glucose tolerance subjects.**

Methods: **Sixteen subjects with body mass index between 27-38 kg/m² were followed monthly for 16 weeks and treated with metformin (1 g/day) and dietary prescription for low-glycaemic index diet with energy reduction of 25-30% their total energy expenditure. Glucose metabolism, lipid profile, anthropometric and body composition, and food intake parameters were measured before and after the treatment. Paired t-tests/Wilcoxon tests were used to compare differences from baseline, with a statistical significance criterion of $p \leq 0.05$.**

Results: **There were significant reductions in anthropometric and body composition parameters, decrease in HOMA2-%β and triglycerides concentrations, and increase in Cederholm index. These results show enhanced peripheral insulin sensitivity and preservation of pancreatic beta-cell function.**

Conclusion: **Calorie-restricted low-glycaemic index diet and metformin was benefit to metabolic and anthropometric parameters in overweight/obese subjects with impaired glucose tolerance.**

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Key words: *Glycemic index. Caloric Restriction. Glucose. Metformin. Type 2 diabetes mellitus.*

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MEJORA DE LA RESPUESTA METABÓLICA DESPUÉS DE 16 SEMENAS DE DIETA CON RESTRICCIÓN CALÓRICA Y BAJO ÍNDICE GLUCÉMICO JUNTO CON METFORMINA EN SUJETOS CON INTOLERANCIA A GLUCOSA

Resumen

Objetivo: **Este estudio analizaba los efectos metabólicos del consejo dietético de seguir una dieta con restricción calórica y un índice glucémico bajo junto con Metformina en individuos con sobrepeso / obesidad y tolerancia alterada a la glucosa.**

Métodos: **Se siguió mensualmente durante 16 semanas a 16 individuos con un índice de masa corporal entre 27-38 kg/m² y se les trató con Metformina (1 g/día) y una prescripción dietética con un índice glucémico bajo y una reducción del energía del 25-30 ¡% de su gasto energético total. Se midieron el metabolismo de la glucosa, el perfil lipídico, la composición antropométrica y corporal y los parámetros de consumo de alimentos antes y después del tratamiento. Se emplearon las pruebas t pareadas y de Wilcoxon para comparar las diferencias con respecto al basal, con un criterio de significación estadística de $p \leq 0,05$.**

Resultados: **Hubo reducciones significativas en los parámetros de composición corporal y antropométricos, una disminución en las concentraciones de HOMA2-% y e triglicéridos y un aumento del índice de Cederholm. Estos resultados muestran una mejora de la sensibilidad periférica a la insulina y una conservación de la función de las células beta pancreáticas.**

Conclusión: **la dieta con restricción calórica y un índice glucémico bajo junto con Metformina fueron beneficiosas para los parámetros metabólicos y antropométricos en individuos con sobrepeso/obesidad y una tolerancia a la glucosa alterada.**

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Palabras clave: *Índice glucémico. Restricción calórica. Glucosa. Metformina. Diabetes mellitus tipo 2.*

Introduction

Obese subjects show progressive increases in glucose and insulin post-prandial responses, which lead to impairment glucose tolerance (IGT) and an increased risk of type 2 diabetes mellitus (T2DM)[1]. First-line intervention for the treatment of obesity and T2DM involves lifestyle modification, including weight management, a healthy diet, and, when necessary, medication[2]. Calorie-restricted diets show improvement in anthropometric and glucose tolerance parameters[3], and low-glycaemic index (low-GI) diet contributes to a lower glucose response and is associated with reduced insulin demand, favouring adequate glycaemic control, lipid profiles, and body composition[4]. Metformin is a potent antihyperglycaemic agent recommended as the first-line oral therapy for T2DM[5] and used to decrease the rate of conversion from IGT to T2DM[6].

Research on low-GI diets has been widely performed; however, the combination with metformin has been poorly investigated. Previous investigations testing metformin in combination with low-glycaemic index foods in T2DM subjects[7,8], women with polycystic ovary syndrome[9] or modified diet in women[10] concluded that it may be an effective adjunct to dietary intervention in the treatment of pre-obese/obese with or at risk of T2DM. The study aims to evaluate the effects of a calorie-restricted low-GI diet combined with metformin on metabolic and body composition parameters of overweight/obese type 1 subjects with impaired glucose tolerance. The aim is to contribute to the information on how the combined treatment helps to improve insulin sensitivity.

Methods

Subjects

The sample size was established considering the CI as the main response variable, assuming 80% power and a 5% significance level. A total of 15 subjects were determined as necessary[11].

This study involved the participation of 18 subjects recruited through public advertisements. The inclusion criteria were adults (19-50 y), of both sexes, with IGT (glycaemia ≥ 7.8 - < 11.1 mmol/L and insulin > 277.8 pmol/L) and body mass index (BMI) between 25 and 40 kg/m², non-smokers, not pregnant or lactating, no diagnosis of any metabolic diseases, and not under medication or therapeutic diets, except for oral contraception in the women.

The protocol of this study was approved by the Ethics Committee in Human Research of the Faculty of Health Sciences at the University of Brasília, Brazil (n°035/2004). All of the volunteers signed a written informed consent form.

Study design

On the first visit, the subjects arrived at the laboratory between 0730 and 0800 hours after a 12-hour overnight fast. Height, waist circumference, body weight, and body composition were recorded and a 2-hour oral glucose tolerance test (OGTT) was administered. The subjects were enrolled for 16 weeks with a monthly follow-up visit (total of 5 visits) to verify adherence and to adjust the dietary treatment and to receive the metformin. The metformin was donated by the Medley Laboratory (São Paulo, SP, Brazil). The dose of metformin was 1 g/day divided in two doses (500 mg) taken with breakfast and dinner.

Dietary counselling

The assessments of physical activity were performed using a short version of the International Physical Activity Questionnaire (IPAQ)[12], and was estimated[13] corrected by the appropriate Physical Activity Level (PAL) of each subject[14]. The dietary energy reduction was set between 25 and 30% of the total energy expenditure, and the dietary macronutrient percentages were set according to the acceptable macronutrient distribution ranges[12]. The dietary energy was distributed by food groups according to the portions defined by the Brazilian food pyramid[14]. A food-grouped portion size equivalent table was created with selected plant-based carbohydrate-fiber foods to help the subjects adhere to the diet.

The dietary adherence was computed for the subjects who completed 5 visits, did not eat more than the proposed dietary energy, consumed 4 - 6 meals per day and had fibre intake 60-70% of the dietary reference value[13].

Food intake assessment

Food intake was assessed by two 24-hour recalls (R24h) at baseline period and five R24h at each follow-up interview during the study intervention. To ensure accuracy, subjects were shown a photographed food portion guide and household items to estimate the food portions consumed. Dietary data were analysed using Nutrition Data System for Research software (version 2011, University of Minnesota, Minneapolis, MN) with inclusions of typical Brazilian food preparations based on standardized recipes. Multiple Source Method was used to estimate the usual dietary intake adjusted by within-person variability[16]. The data provided represents the baseline and intervention usual dietary intake.

Anthropometric and body composition assessment

Body weight was assessed using an electronic platform scale (Plenna, São Paulo, Brazil) with range of 0-

150 kg and precision of 0.1 kg. Height was measured using a stadiometer (Alturaexata, Belo Horizonte, Brazil) with a range of 0-210 cm and a precision of 0.1 cm. BMI was computed and classified[17], and body fat percentage was measured by tetrapolar electrical bioimpedance (Quantum II-RJL Systems, Clinton Township, MI, USA)[18]. The waist circumference was measured and classified according to NCEP-ATP III[19].

Physical activity level

The short version of IPAQ was adapted to obtain physical activity description at initial and monthly return visits[12]. A partial activity ratio was calculated by time spent on each activity multiplied by the energy costs of activities, expressed as multiples of basal metabolic rate. The partial results were added and divided by 24 to give the PAL by the FAO/WHO/UNU factorial approach[20].

Biochemical analyses

Capillary blood samples were taken to verify the fasting state for the OGTT using a glucometer (Roche Diagnostics, Mannheim, Germany). At baseline and at 16 weeks, blood samples were collected after a 12-hour overnight fast. The concentrations of serum insulin, triglycerides, total cholesterol (TC), HDL-c, LDL-c, and VLDL-c were determined. Serum insulin was measured by electrochemiluminescence (Elecsys 2010, Roche Diagnostics, Mannheim, Germany) and plasma glucose by the glucose oxidase method (Immulite 2000, DPC, Los Angeles, USA). The TC, HDL-c fraction, and triacylglycerol were measured using enzymatic colorimetric kits (Labtest Diagnostica S.A., Belo Horizonte, Brazil). The fractions of LDL-C and VLDL-c were calculated using the Friedewald equation[21]. Four millilitres of blood were collected in ice-cooled EDTA-plasma vacutainers to determine the value of glycated haemoglobin (HbA1c) by high performance liquid chromatography method[22].

Oral glucose tolerance and insulin sensitivity tests

A 2-hour OGTT was performed after an overnight fast and blood samples were drawn at baseline, 30, 60, 90, and 120 minutes. The incremental area under the curve (AUC) for glucose and insulin was calculated excluding any value below the baseline value using the trapezoidal method[23]. Values for the homeostasis model assessment insulin resistance (HOMA2-IR), HOMA β-cell function (HOMA2-%β), HOMA insulin sensitivity (HOMA2-%S)[24], and the Cederholm index (CI)[25] were calculated to determine insulin resistance, β-cell function, and insulin sensitivity.

Statistical analysis

The Kolmogorov-Smirnov test was applied to verify the normality of the variables and residual data plots were examined to determine the homogeneity of variance. Paired t-tests/Wilcoxon tests were used to compare differences between baseline and 16 weeks intervention. Pearson's correlation coefficient was used to evaluate linear dependence between fat loss against the change (final-initial) in metabolic and anthropometric parameters. All statistical analyses were performed using the SAS software package, version 9.1 (SAS Institute Inc., Cary, NC, USA), with a statistical significance criterion of $p \leq 0.05$, two-tailed.

Results

Subject characteristics

Of the 18 subjects recruited, 16 successfully completed the intervention (9 male, mean age 34.6 ± 7.0 years old, and BMI 31.6 ± 2.9 kg/m^2). The individuals maintained the pattern of physical activity during the intervention (initial PAL 1.36 ± 0.09 and final PAL 1.39 ± 0.06, $p = 0.58$).

Clinical and biochemical parameters

The intervention resulted in significant reductions from baseline values in weight ($p < 0.0001$), BMI ($p < 0.0001$), waist circumference ($p < 0.0001$), body fat ($p < 0.0001$) and increases in fat-free mass ($p < 0.0001$). Significant decreases in HOMA2-%β ($p = 0.04$), TAG and VLDL-c (both $p = 0.03$), and increased CI ($p = 0.04$) values were observed (table I). There was no significant modification in AUC glucose and AUC insulin, but there was a significant ($p = 0.03$) reduction in insulin concentration at 120 minutes (fig. 1).

There were significant correlations between fat loss (kg) and change (**Δ**) in body weight (r = 0.65; p = 0.006), **Δ**BMI (r = 0.66; p = 0.006), and tendency for **Δ** HOMA2-%S (r = 0.46; p = 0.09). Furthermore, a significant negative correlation was observed between fat loss and **Δ** insulin (r = -0.56; p = 0.04) and **Δ** AUC glucose (r = -0.50; p = 0.04) (table II).

Food Intake

There were improvements in the usual nutrient intake during the study intervention compared to baseline period. There were reductions in energy ($p < 0.001$), protein ($p = 0.02$), lipids ($p = 0.004$), carbohydrate ($p < 0.001$), PUFA ($p < 0.001$), and cholesterol ($p = 0.005$) intake, and increase in fibre ($p = 0.002$) intake. Furthermore, glycaemic index and glycaemic load determination of the usual intake confirmed the expected

Table I
Anthropometric, body composition and biochemistry variables analysed at baseline and after 16 weeks of dietary treatment and metformin

	Baseline		*16 weeks*			
Variables	*Mean*	*SD*	*Mean*	*SD*	Δ	*p*
Weight (kg)	86.62	14.00	82.28	12.98	-4.34	<0.0001
BMI (kg/m²)	31.57	2.88	30.03	2.94	-1.54	<0.0001
Waist (cm)	96.59	7.93	91.73	7.18	-4.86	<0.0001
Body fat (%)	33.11	7.04	30.58	7.22	-2.53	<0.0001
FFM (%)	66.89	6.76	69.42	7.14	2.53	<0.0001
HbA1c (%)	6.24	0.68	6.04	1.05	-0.2	0.62
Glucose (mmol/L)	4.68	0.52	4.69	0.46	0.01	0.96
AUC Glucose	7390	2232	6894	2438	-496	0.38
Insulin (pmol/L)	88.55	49.93	77.57	55.00	-10.98	0.45
AUC Insulin	13267	8226	10219	6065	-3048	0.19
CI	0.008167	0.001864	0.009306	0.001765	0.001139	0.04
HOMA2-IR	1.53	0.60	1.23	0.60	-0.30	0.09
HOMA2-%β	158.09	75.44	124.05	42.85	-34.05	0.04
HOMA2-%S	75.17	30.51	96.29	38.33	21.13	0.10
TAG (mmol/L)	1.78	0.80	1.47	0.55	-0.31	0.03
TC (mmol/L)	4.76	1.20	4.50	0.88	-0.26	0.47
LDL-c (mmol/L)	2.64	1.03	2.47	0.77	-0.17	0.56
VLDL-c (mmol/L)	0.82	0.36	0.68	0.25	-0.14	0.03
HDL-c (mmol/L)	1.30	0.31	1.35	0.22	0.05	0.56
TC/HDL-c	3.77	1.24	3.34	0.48	-0.43	0.14

Statistical analysis: Paired t-test/Wilcoxon test was applied to analyse baseline *vs* 16 weeks. FFM, fat-free mass; HbA1c, glycated haemoglobin; AUC Glucose, area under the glycaemic curve; AUC Insulin, area under the insulinaemic curve; CI, cederholm index; HOMA2-%β, homeostasis model assessment β-cell function; HOMA2-IR, homeostasis model assessment insulin resistance; HOMA2-%S, homeostasis model assessment insulin sensitivity; TAG, triglyceride; TC, total cholesterol; TC/HDL-c, total cholesterol / high-density lipoprotein cholesterol ratio.

change in the dietary pattern imposed by the intervention (table III).

Discussion

Hyperglycaemia maintained over a period of years leads to β-cell failure which causes impaired glucose tolerance and type 2 diabetes[26]. Low-glycaemic index diet and metformin improve glucose metabolism parameters and prevent the development of T2DM among IGT subjects[27,28].

In the present study the combined intervention was positive for weight loss and improvement in HOMA2-%β and the CI. The results were highly significant for body composition and significant for the TAG and VLDL concentrations, which indicate a positive progression of the lipid profile. Furthermore, we observed a significant correlation between fat loss and changes in body weight, BMI, and negative correlation between fat loss and changes in insulin and AUC glucose.

Obesity, and especially visceral adiposity, is associated with an increased risk of insulin resistance and type 2 diabetes. Excess adipose tissue releases increased amount of non-esterified fatty acids, glycerol, hormones, pro-inflammatory cytokines and other factors that are involved in the development of insulin resistance. A chronic excess of non-esterified fatty acids results in an increase in fat deposits in muscle and liver and increased metabolites, such as diacylglycerol and ceramide, which activate isoforms of protein kinase C, impairing cellular insulin signalling. Chronically high lipid levels affect the beta cell function and insulin sensitivity leading to hyperglycaemia[29,30]. Fat loss therefore leads to improvement in beta cell function and glucose metabolism.

Lifestyle modification (diet plus physical activity) and metformin were recommended as the first-line therapy for IGT and T2DM. Results from the U. S. Diabetes Prevention Program trial[31], which evaluated over 3000 subjects divided in three treatments, showed that in the metformin group (1.7 g/day), diabetes risk, insulin sensitivity, and beta-cell function at 1 year were intermediate between those in the intensive lifestyle and placebo groups. The combination of dietary intervention (calorie-restricted low-glycaemic index diet) and metformin was able to improve insulin parameters in a shorter treatment period, without the intensive lifestyle modification (diet and physical activity). Dietary changes and exercise are factors known to positively affect improvements in insulin sensitivity[32], but these intense lifestyle changes are difficult to adhere to[32,33]. Also, since the improvement in insulin sensitivity due to exercise rapidly disappears after its cessation[34], a continued treatment scheme is still being sought. The Indian Diabetes Prevention Programme

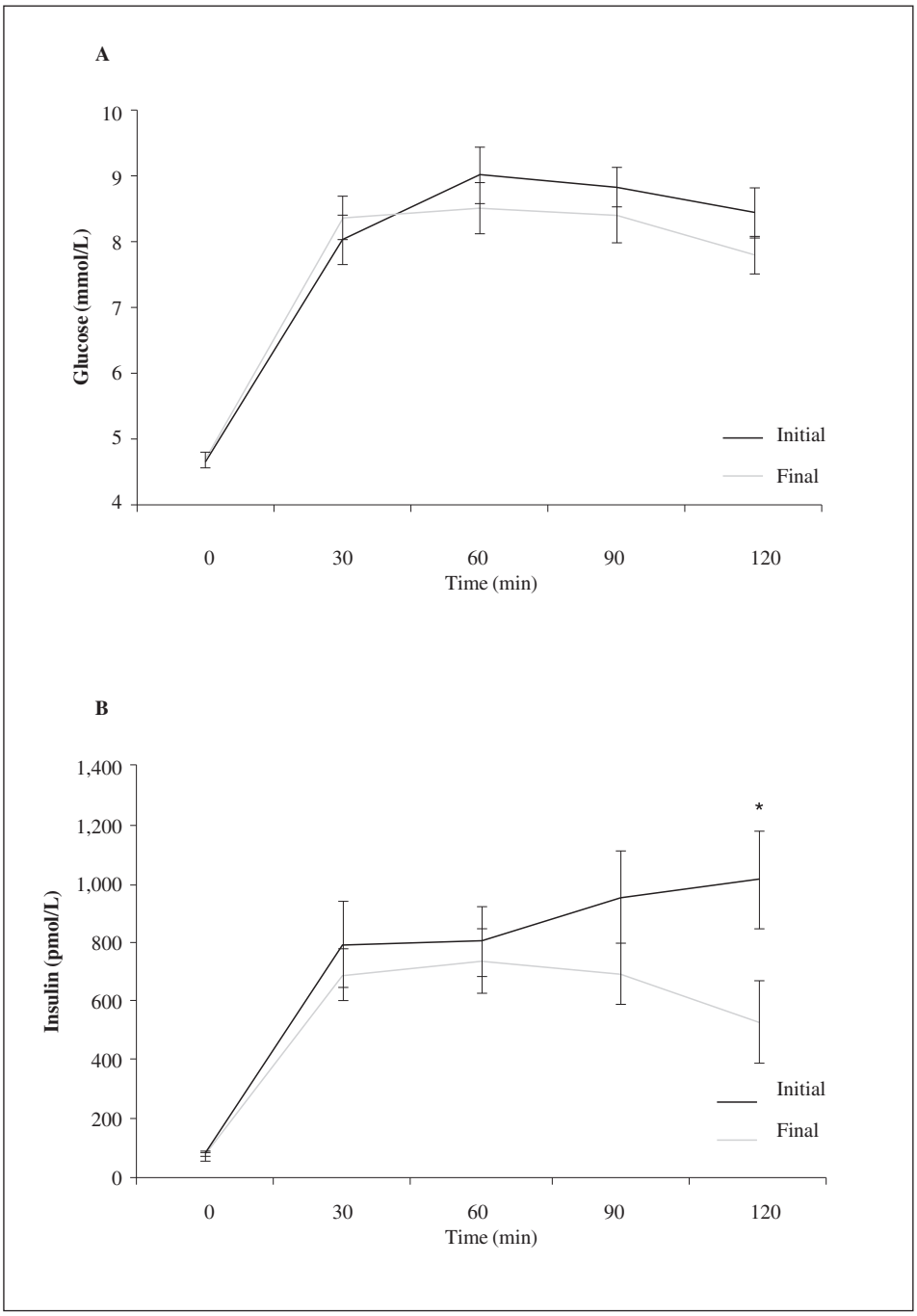

*Fig. 1.—Two hours postprandial glycaemic **(A)** and insulinaemic response **(B)** to a calorie-restricted low-glycemic index diet and metformin (LGI + MET).*
*Data are expressed as the mean ± standard error. * $p \leq 0.05$.*

followed a similar design to the U.S Diabetes Prevention Program, but it also included a combined lifestyle change and metformin group (0.5 g/day)[33]. The addition of metformin to lifestyle modification (diet and exercise) in the Indian study did not enhance the effectiveness. One possible explanation is the low dosage of metformin used in the trial. So the question related to the combined treatment is still debatable.

The mechanisms of action of metformin include a decrease glucose output from the liver and increased glucose uptake from peripheral tissues[35]. It also reduces postprandial glycaemic peaks and improves insulin action, presenting anti-oxidative and anti-inflammatory actions[36]. Several studies showed that the beneficial metabolic effects of metformin involve AMP-activated protein kinase (AMPK)[5,36-39]. Moreover, metformin has an anorexic effect which involves AMPK[37,38], inhibition of the hypothalamic orexigenic peptides, neuropeptide Y and agouti-related protein[40] and incretin[38]. AMPK activation increases glucose uptake by skeletal muscles, increases lipid catabolism and leads to control of the appetite and satiety by the hypothalamus[40]. The AMPK system may be partly responsible for the health benefits of exercise. This enzyme is a key player in the

Table II
Pearson correlation among Δ fat loss (final-initial) (kg) after 16 weeks of dietary treatment and metformin

Δ *Variables (final-initial)*	*r*	*p value*
Weight (kg)	0.65	0.006
BMI (kg/m²)	0.66	0.006
Waist (cm)	0.32	0.23
HbA1c (%)	-0.41	0.15
Glucose (mmol/L)	-0.03	0.92
AUC glucose	-0.50	0.04
Insulin (pmol/L)	-0.56	0.04
AUC insulin	-0.10	0.71
CI	0.34	0.19
HOMA2-%β	-0.26	0.38
HOMA2-IR	-0.37	0.19
HOMA2-%S	0.46	0.09
Triglycerides (mmol/L)	-0.20	0.45
Total cholesterol (mmol/L)	-0.35	0.18
LDL-c (mmol/L)	-0.35	0.18
VLDL-c (mmol/L)	-0.19	0.49
HDL-c (mmol/L)	-0.14	0.61

HbA1c, glycated haemoglobin; AUC glucose, area under the glycemic curve; AUC insulin, area under the insulinemic curve; CI, cederholm index; HOMA2-%β, homeostasis model assessment β-cell function; HOMA2-IR, homeostasis model assessment insulin resistance; HOMA2-%S, homeostasis model assessment insulin sensitivity.

development of new treatments for obesity, T2DM, and the metabolic syndrome[37,39,41].

The measurements of peripheral and central actions of metformin on insulin control of glycaemia are important. Among the various methods to evaluate insulin sensitivity from fasting measurements HOMA is the most commonly used. HOMA is indicative of the balance between hepatic glycogenesis and gluconeogenesis when fasting[42]. On the other hand, the CI is based upon a physiological model and estimates the mean enhancement of glucose effectiveness due to plasma insulin during the OGTT, correcting for the total body glucose space[25]. Generally, HOMA is commonly taken to represent central or hepatic insulin sensitivity, and the CI is indicative of peripheral insulin sensitivity. Under the combined dietary-metformin intervention we showed an increase in the peripheral insulin sensitivity with an increase in CI. There was a significant reduction in HOMA2-%β and a 20% reduction in HOMA2-IR (1.53 ± 0.60 to 1.23 ± 0.60) and 28% increase in HOMA2-%S (75.17 ± 30.51 to 96.29 ± 38.33), which did not reach statistical significance but is physiologically relevant. These responses are compatible with the actions of metformin on muscle mass through the AMPK system described above.

Furthermore, we observed significant correlation between fat loss and changes in body weight and BMI. These results show that higher fat loss is related to improvement in these parameters, which is compatible with the activation of the AMPK by diet and metformin[39]. On the other hand, fat loss was negatively correlated with change in insulin and AUC glucose, which means that a poor body fat loss is correlated to a decrease in these parameters. This result is unexpected and should be investigated using a multiple regression model that permits an evaluation of mechanism controlling glucose uptake under the effects of metformin.

This study has many limitations, as the therapy was tested in a small number of subjects in a pairwise, short follow-up protocol. Furthermore the study design is weak because was not included a true (untreated) control group, so it is not possible to determine the real effects of the metformin in the analyzed variables compared with a group who received no drug. However, these results contribute to the perspective of combined treatment of low-GI benefits associated with a relatively low dose of metformin, as there is a lack of data on the combination of lifestyle intervention and antidiabetic agents in pre-diabetic patients[43].

In conclusion, we showed the benefits of calorie-restricted low-glycaemic index diet with metformin on metabolic and anthropometric parameters in overweight/obese IGT subjects. The increase in Cederholm index is compatible with improvement in peripheral insulin sensitivity, associated with an improved response of the beta cell. However, longer duration follow-up trials are necessary to understanding the metabolic

Table III
Usual nutrient intake analysed at baseline and during the 16 weeks of dietary treatment and metformin

	Baseline		*16 weeks*		
Variables	*Mean*	*SD*	*Mean*	*SD*	*p*
Energy (Kcal)	2138	683	1583	351	< 0.001
Protein (g)	96.7	32.0	78.4	24.1	0.02
Lipids (g)	82.4	32.3	57.7	14.3	0.004
Carbohydrate (g)	254.4	76.2	191.0	34.4	< 0.001
Fibre (g)	14.3	3.8	18.9	3.8	0.002
PUFA (g)	18.2	5.1	12.2	3.5	< 0.001
Cholesterol (mg)	302.9	161.9	196.2	60.5	0.005
Glycemic index	60.7	2.1	55.2	2.7	< 0.001
Glycemic load	146.3	42.5	95.3	21.2	< 0.001

Statistical analysis: Paired t test/Wilcoxon test was applied to analyse baseline *vs* 16 weeks. PUFA, polyunsaturated fatty acid.

effects and effectiveness of low-GI diets and metformin in glycaemic and insulinaemic responses to prevent pre-diabetes from progressing to T2DM.

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